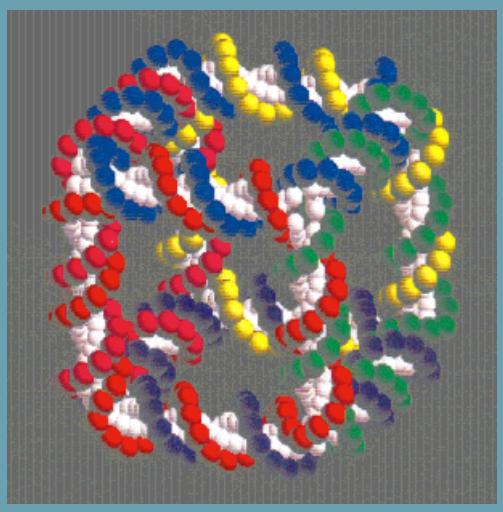

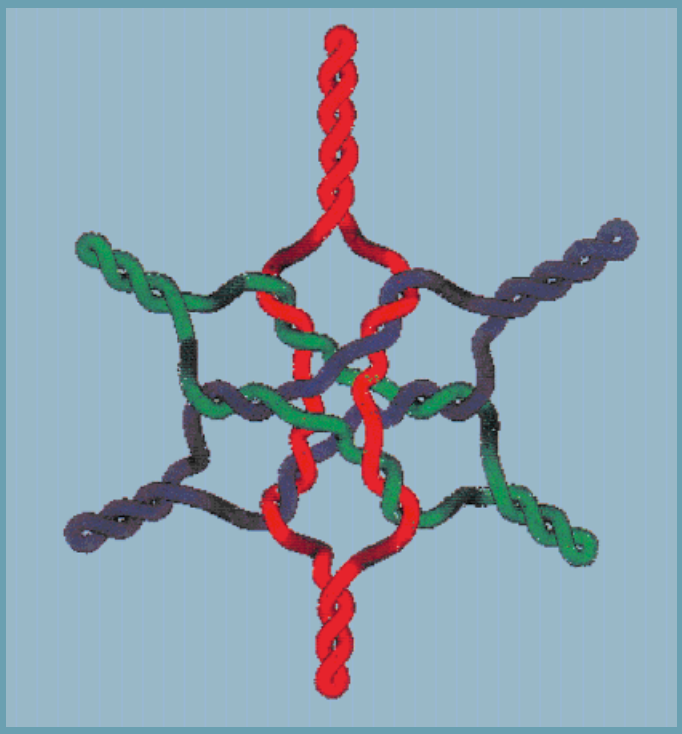

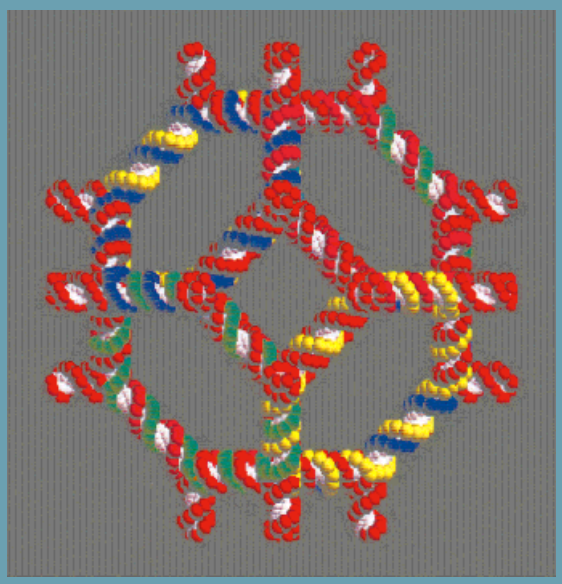

Three aesthetically pleasing topological target structures resulting from DNA nanotechnology (from top to bottom): a cube, Borromean rings, and a truncated octahedron.

These molecules are constructed by applying the assembly methods of biotechnology to stable branched DNA molecules.

Nucleic Acid Nanostructures and Topology

Nadrian C. Seeman*

DNA is well known as the genetic material of all living organisms. However, the properties that allow it to serve so well in that role also can be exploited for chemical ends. Indeed, one of the most promising branches of nanotechnology is predicated on this genetic molecule. It is possible to make stable branched DNA molecules that can be combined through the specificity of cohesion of two ("sticky") ends with complementary overhangs to direct the construction of stick figures. Molecules whose edges consist of double-helical DNA have been constructed which show the connectivities of a cube and of a truncated octahedron. Two-dimensional arrays with programmed structural features have also been built. The topological properties of DNA have been exploited to produce specific knots and Borromean rings. In addition, DNA transitions can be used as the basis of nanomechanical devices. There is far more to DNA than a repository for genetic information!

Keywords: catenanes · DNA structures · mechanical bonds · nanostructures · topochemistry

1. Introduction

1.1. DNA Nanotechnology

The term "nanotechnology" has become a word encountered frequently in chemistry. There are many interpretations to this word, but the most visible goal of nanotechnology is to establish the same level of control over the manipulation and assembly of real molecules and molecular groupings that molecular graphics has over the manipulation and assembly of molecular models. There have been two key inspirations for this approach to very ambitious chemistry: The first was Feynman's famous talk, entitled "There's Plenty of Room at the Bottom".[1] He suggested that it would be possible to make machines that could make replicas of themselves on a somewhat smaller scale; the ultimate application of this procedure would be very tiny factories that could be packed densely. The second inspiration has been our growing knowledge of the ways that biological systems arrange their structural components, primarily by self-assembly on a supramolecular scale; hence, this second approach to nanotechnology is biomimetic. Feynman's approach has engendered the "top-down" methodology, best exemplified today by molecular assemblies that have been arranged by using scanning probe microscopes.[2] By contrast, biomimetic nanotechnology is "bottom-up", in that individual components are designed to assume particular tertiary structures, and ultimately to self-assemble into quaternary structures and arrays.

Top-down nanotechnology is characterized at this stage by elegance and by the ability to deal with substances of largely arbitrary composition. Its key limitation is its lack of parallelism; only small numbers of objects can be assembled at once. The bottom-up approach has a number of drawbacks, but it has the primary advantage of enormous parallelism; even chemistry done with a picomole of material results in more than 100 billion copies of the product species. Biomimetic nanotechnology cannot make use conveniently of intervention by the scanning probe microscopist; hence it has an additional burden, the design of molecules that fold correctly and reliably into their proper shapes. We understand this type of design best in those systems composed of biological macromolecules. This is a very restricted set of species, and their behavior is largely limited to aqueous systems. Nevertheless, we have better control over the folding of nucleic acids and, to a lesser extent, proteins than over most other polymers on the nanoscale. The design of a protein and accurate prediction of protein folding remain key challenges in the area, although striking progress is being made.[3] Nucleic acids are much simpler, and therefore we have a much better grasp of their folding. Here, we will dwell on nucleic acid nanotechnology, primarily DNA nanotechnology.

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1.2. Key Targets for DNA Nanotechnology

There are many possible arrangements of matter that one might wish to assemble from DNA. The original motivation for this program was, from the point of view of crystallography, the desire to arrange DNA in a periodic array; the idea was to provide a scaffold on which to arrange other macromolecules that are intractable to conventional crystallization procedures.[4] A logical extension of this strategy is to use the same type of scaffolding to arrange molecular electronic components, thereby creating dense memories.[5] Neither of these goals has been achieved yet, but we will describe progress towards them.

When one thinks of chemical species derived from DNA, it is natural to ask whether they could be produced more economically by means of biologically based reproduction, either in vivo or by the polymerase chain reaction (PCR), which makes effective use of the cell's DNA replication enzymes.[6] Owing to topological considerations, these methods are likely to be applied most effectively to DNA knots. We will discuss work on DNA knots and other topological targets in Section 4.

Finally, DNA is a molecule that is known to undergo large structural transitions. These transitions present an opportunity to convert stable DNA species into nanomechanical devices. Two key transitions have been explored, and nanomechanical motion has been derived from each of them. These recent studies will be covered in Section 5.

2. DNA as a Construction Material

2.1. DNA as a Component for Molecular Design

DNA is well known as the genetic material of all living organisms. Indeed, its double-helical structure has become one of the cultural symbols of our civilization in much the same way that we associate previous societies with the Hanging Gardens of Babylon, the Colossus of Rhodes, or the Great Wall of China. The key feature that makes DNA so well suited to its biological role is the complementarity relationships that form hydrogen-bonded Watson – Crick base pairs:[7] Adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C). These classical interactions represent the acme in molecular recognition: From the standpoint of molecular design, DNA has the most predictable and readily programmed intermolecular interactions of any molecular species on its scale.

In general, it is very difficult to predict the preferred geometry by which two macromolecules will associate; extensive calculations are necessary to derive a set of potentially favorable interaction geometries between two molecules. However, in the case of DNA, these difficulties reduce to the base-pairing rules. Two complementary strands under appropriate conditions will form a double helix with Watson – Crick base pairs, in preference to any other possible interactions. This behavior of DNA has been exploited for a quarter of a century by biotechnologists. Two molecules with complementary overhanging sequences, called "sticky ends", will associate with each other preferentially. The sticky-ended association of DNA molecules by hydrogen bonding is illustrated in Figure 1. The local three-dimensional structure

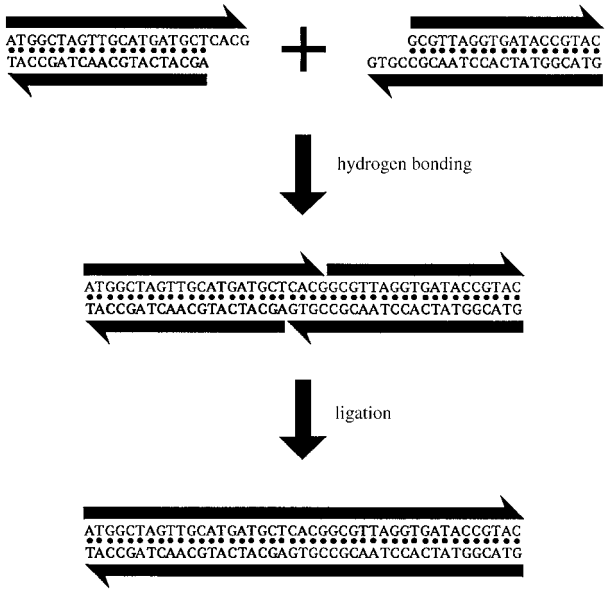

Figure 1. Sticky-ended cohesion and ligation. Top: two linear double-helical molecules of DNA. The antiparallel backbones are indicated by the black lines terminating in half-arrows. The half-arrows indicate the 5′ →3′ directions of the backbones. The right end of the left molecule and the left end of the right molecule have single-stranded extensions ("sticky ends") that are complementary to each other. Middle: under the proper conditions, the extensions bind to each other specifically by hydrogen bonding. Bottom: the extensions can be ligated to covalency by the proper enzymes and cofactors.

Nadrian C. Seeman was born in Chicago in 1945. Following a B.S. in biochemistry from the University of Chicago, he received his Ph.D. in biological crystallography from the University of Pittsburgh in 1970. His postdoctoral training, at Columbia University and the Massachusetts Institute of Technology, emphasized nucleic acid crystallography. He obtained his first independent position at the State University of New York in Albany, where his frustrations with the macromolecular crystallization experiment led him one day to the campus pub. There, he realized that the similarity between six-arm DNA branched junctions and the periodic array of flying fish in M. C. Escher's "Depth" might lead to a rational approach to crystallization and structural control on the nanometer scale. He has been trying to implement this form of DNA nanotechnology ever since, for the last ten years at New York University. Further information is available at http://seemanlab4.chem.nyu.edu in internet.

of the base-paired region is known to be B-DNA,[8] the familiar structure assumed by DNA in solution. Thus, not only can preferred affinity between DNA molecules be programmed and predicted, but so can the local structure of the intermolecular complex. Figure 1 also illustrates that it is possible to seal the two molecules together by using DNA ligase to catalyze the formation of phosphoester bonds in the backbone.[9]

In addition to the simplicity of directing its interactions, there are other advantages to DNA as a medium for nanoconstruction. The needs of biotechnology have resulted in extremely convenient automated phosphoramidite chemistry for the synthesis of arbitrary sequences.[10] In addition, many exotic phosphoramidites are commercially available to simplify the derivatization, detection, analysis, and purification of synthetic DNA molecules. It is also easy to manipulate DNA, because there is a large battery of commercially available DNA-modifying enzymes: These include restriction enzymes that cleave DNA at specific sequences, ligases that join ends, exonucleases that digest linear but not cyclic strands, and topoisomerases that catalyze operations in which strands are passed through each other (strand passage). DNA also has an external code[11] that can be read by proteins. Furthermore, as a polymer, DNA is both chemically stable[12] and relatively stiff, having a persistence length of roughly 500 Å under conventional conditions.[13] All of these advantages argue strongly for working with a DNA backbone, in contrast to nonstandard backbones,[14] which are not known to be substrates for modifying enzymes. The difficulties we experienced in a recent project involving RNA molecules[15] have reinforced our preference for DNA.

2.2. DNA Branched Junctions

At this point, the reader may be thinking that it is perhaps very convenient to tack DNA molecules together by programming sticky ends, but to what end? A long polymer of DNA has obvious biological utility, but what chemical targets does this open up? The answer is to be found in one of the subtle processes of biology, genetic recombination. The basic molecular feature of recombination is the interaction of two pieces of DNA to yield new genetic material. A key intermediate in recombination is the Holliday junction,[16] in which four connected double helices flank a branch point. The sequence that flanks the branch point of a Holliday junction is twofold symmetric; the symmetry enables the branch point to relocate within the region of symmetry by an isomerization known as branch migration.[17] Fortunately, it is possible to design synthetic sequences lacking this symmetry; thus, one can make branched molecules, termed junctions, with fixed branch points.[4]

The ability to direct DNA molecules to form branched structures adds a new geometrical element, the vertex, to DNA structural chemistry. The helix axes of linear DNA molecules are equivalent to line segments in geometry. By joining them as illustrated in Figure 1, one can make longer lines or circles, perhaps knots and catenanes. However, by including vertices among the components, objects and networks[18] become potential targets; these are often described as *N*-connected species,[18] where *N* refers to the number of edges that meet at a vertex. The objects will be stick figures, whose edges consist of double-helical DNA, and whose vertices correspond to the branch points of the junctions. This concept is illustrated in Figure 2, where four four-arm branched junctions are assembled into a quadrilateral. The junctions each contain four sticky ends, X and its complement X′, Y and its complement Y′. Each of the four junctions coheres with its neighbors by means of these sticky ends. In addition, there are sticky ends on the outside of the quadrilateral, so one could imagine extending this structure infinitely to tile the plane.

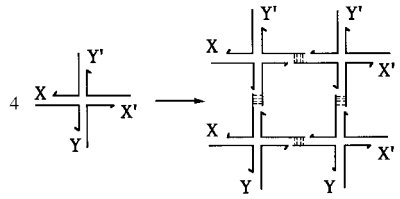

Figure 2. Formation of a two-dimensional lattice from an immobile junction with sticky ends. X and Y are sticky ends, and X′ and Y′ are their complements. DNA ligase can close the gaps.

How does one design DNA sequences to assemble into stable branched molecules? The fundamental assumption of the design is that under conventional conditions the most favorable structure for DNA is the linear double helix.[4, 19] Putting a branch into DNA costs free energy, relative to this favored structure.[20] However, a branch can form if double-helix formation covers the cost of branching. Our implementation of this concept is illustrated in Figure 3. First, to eliminate branch migration,[17] we ensure that the branch point is not flanked by a sequence with twofold symmetry. In

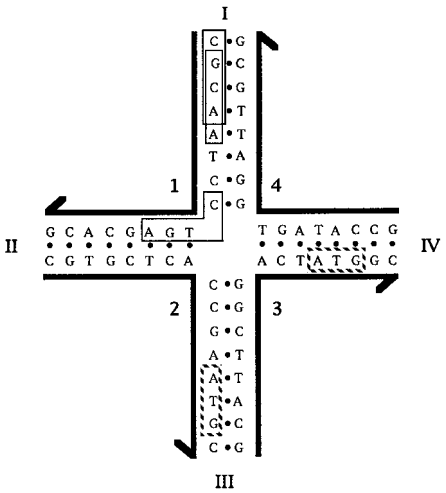

Figure 3. A stable DNA branched junction composed of four strands of DNA (1–4). The arms are labeled with I–IV. The 3′ end of each strand is indicated by a half-arrow. Each strand is paired with two other strands to form a double-helical arm. There is no homologous twofold sequence symmetry flanking the central branch point which stabilizes its position. Tetrameric elements are in solid boxes, and trimeric elements in dashed boxes.

addition, we divide the sequence of each strand into a series of overlapping elements. The elements shown are tetramers, so that each of the 16-mer strands is treated as a set of 13 overlapping tetramers. The first two tetramers on strand 1 are boxed: CGCA and GCAA. We insist that each of these tetrameric elements be unique. We also forbid the complement to any element that spans a bend: For example, there is no TCAG to complement the tetramer CTGA at the bend of strand 1. Using this approach, competition with the target duplex structures (octamers of base pairs in each arm) will come only from trimers, such as the ATG sequences in the dashed boxes. In principle these ATG sequences could pair with the wrong CAT, but in practice this type of problem is not seen; hence the algorithm is effective in producing target secondary structures.

Tetramers provide a vocabulary of 240 non-self-complementary sequences (4^4 less 16 self-complementary sequences), from which 52 elements can be selected to satisfy the conditions. If trimer elements were used, competition would only be from dimers, but there would be a vocabulary of only 64 elements (4^3) to supply the 56 trimers needed for the structure, which becomes difficult. It is clear that one must use longer elements (of length N) with larger structures. If the arms of the branched junctions do not get appreciably longer, then competition from $(N-1)$-mers may become significant, for example, pentamers if hexamer elements ($N=6$) are used. Although we have not done so, it may prove useful to check $(2N-1)$-mers for near-identity: With tetrameric elements, the algorithm would not be sensitive to the similarity between ATTCGCA and ATTAGCA (6 out of 7 nucleotides). In practice, one must often incorporate sequences into the target molecule that violate these rules: For example, restriction sites are frequently symmetric. We usually incorporate these sites first, and then design the rest of the sequence around them.

The number of arms in a branched DNA molecule is not limited to four. We have made junctions that contain three,[21] five, and six[22] arms; there is no clear limit to the number of arms that can flank a branch point.[23] However, the more arms that are incorporated, the longer the arms must be in order to stabilize the branch point;[22] the reason for this may be the accumulation of negative charge in the region of the branch point. The number of arms flanking a junction establishes the maximum connectivity of the objects or networks that can be constructed from it. For example, three-arm junctions can make a stick cube, but one needs four-arm junctions to make a stick octahedron, five-arm junctions for a stick icosahedron, and six-arm junctions for a simple cubic stick lattice.

2.3. Topology in DNA Constructions

The fact that DNA is a double helix, and not a double ladder, complicates thinking about the molecule, but it is not a serious impediment to working with it. In fact, the differences between helices and ladders open up a number of targets that otherwise would not be particularly suitable for DNA. Nevertheless, the consequences of the topology must be taken into account in experimental design. Figure 4 illustrates this point by showing two different designs for the assembly of the molecule in Figure 2. In Figure 4a, the branch points of the junctions are separated by exactly two turns of DNA. The resulting single strands are a series of cyclic molecules linked together to form molecular chain mail. Cyclic molecules held together by topological bonds are called catenanes. The array in Figure 4b is assembled by the same design, but here the vertices are separated by 1.5 turns of DNA. The resulting single strands form no circles, but correspond to a warp and weft interwoven network.

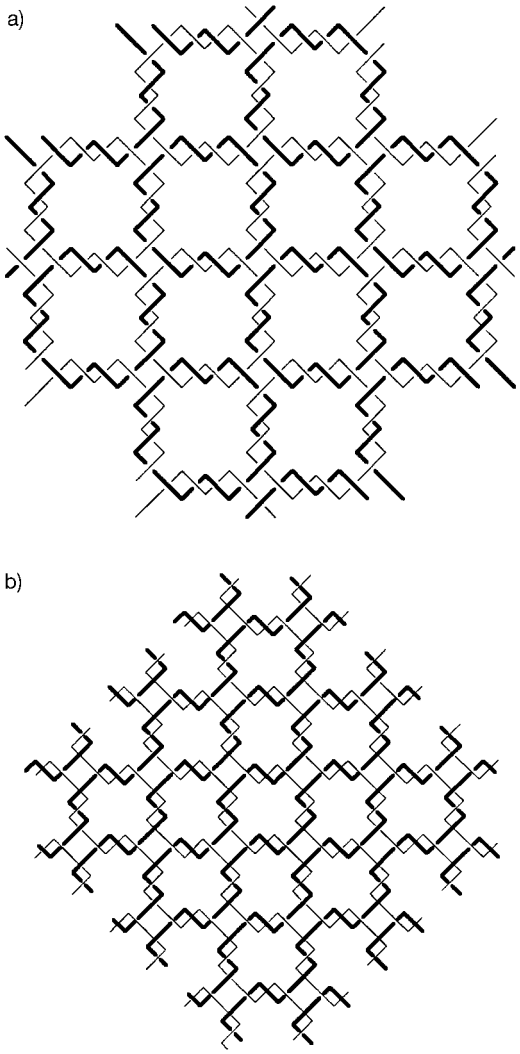

Figure 4. Topological consequences of ligating DNA molecules containing even and odd numbers of DNA half-turns in each edge. These diagrams represent the same ligation shown in Figure 2. However, they indicate the plectonemic (interwound) winding of the DNA, and its consequences. The DNA is drawn as a series of right-angled turns. a) Each edge of the square contains two turns of the DNA double helix. Paired strands are drawn with alternating thick and thin lines. b) Each edge of the square contains 1.5 turns of DNA.

The difference between the integral and half-integral separations of junctions arises repeatedly in the design of DNA objects. In a sense, the double-helical half-turn is the quantum of single-stranded DNA topology. It corresponds to

a crossing or node in a DNA catenane or knot.[*] Sometimes a node is called a "unit tangle".[24] We will see below that the equivalence between a double-helical half-turn and a unit tangle can be very useful in the construction of topological targets. It appears possible to design three-dimensional species whose vertices are separated by nonintegral numbers of half-turns.[25, 26]

2.4. Flexibility of DNA Branched Junctions

The assembly shown in Figure 2 is predicated on two assumptions, the cohesion between complementary sticky ends and the rigidity of the DNA branched junction. Figure 5 illustrates the fact that a variable angle between the arms could result in a different product: The same sticky ends are

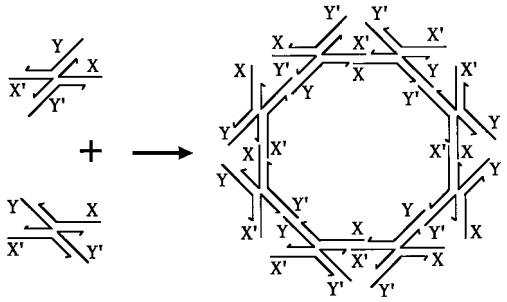

Figure 5. Consequences of variable angles between the arms. The same ligation is shown as in Figure 2, but the angles between the arms are variable. A cyclic complex corresponding to eight molecules is shown; any $4N$ complex could be cyclic in this system.

shown to adopt two conformations; when altered around the cycle, an octagonal figure results. Unfortunately, the angles between the arms of the junction are not fixed. We have investigated the rigidity of three-arm junctions,[21] four-arm junctions,[27] and three-arm bulged junctions[28] in ligation-closure experiments. A typical experiment involving three-arm junctions is illustrated in Figure 6. The junction contains two complementary sticky ends that have been phosphorylated, so that they can be ligated; the blunt end is excluded from the experiment, because it contains no phosphate group. The design of the experiment is important, because one must ensure that a "reporter strand" is available. This is a single strand whose fate reflects the fate of the complex; its size and linear or cyclic character can be ascertained readily by denaturing gel electrophoresis. In Figure 6, the reporter strand is shown labeled with a radioactive phosphate. If two or more junctions are ligated together, the length of the reporter strand increases proportionately. Similarly, if the complex cyclizes (Figure 6, right), then the reporter strand cyclizes. After ligation, the products are applied to a

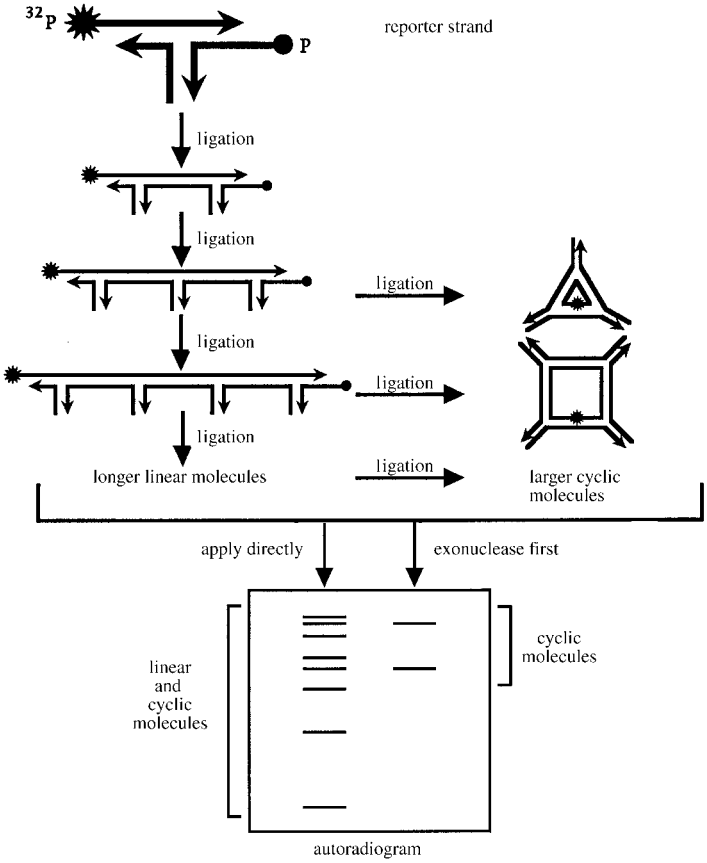

Figure 6. The experimental design used in the ligation assay. The three-arm junction employed in all of the experiments is indicated at the upper left. The 3′ ends of the strands are indicated by arrowheads. The 5′ end of the top strand contains a radioactive phosphate group (starburst), and the 5′ end of the strand below the first and to the right contains a nonradioactive phosphate group (filled circle). The third strand corresponds to the blunt end, and is not phosphorylated. Beneath this molecule are shown the earliest products of ligation, the linear dimer, the linear trimer, and the linear tetramer. The earliest cyclic products are shown on the right, the cyclic trimer and the cyclic tetramer. The blunt ends form the exocyclic arms of these cyclic molecules. Note that in each case the labeled strand has the same characteristics as the entire complex: It is an oligomer of the same multiplicity as the complex, and its state of cyclization is that of the complex; thus, it is a reporter strand. The analysis of the reactions products is shown in the lower part of the figure (see text for further details).

denaturing gel, and an autoradiogram is developed. The lane on the left, whose contents are applied directly to the gel, contains both linear and cyclic reporter strands. However, if the material is treated first with exonucleases (exo III and/or exo I; right lane), only cyclic products remain, because they are resistant to digestion by these enzymes. The unhappy result of these experiments is that all simple branched junctions tested result in a plethora of cyclic products, indicating that they contain variable angles. We will describe below a rigid DNA system, but the major stumbling block to controlling the assembly of DNA objects and lattices is the flexibility of the angles between the arms of branched junctions. This flexibility leads to only topological control over the products, rather than geometrical control; likewise, proofs of synthesis are topological proofs, rather than geometrical characterizations.

[*] A node (or unit tangle) is a crossing between two strands seen when the structure is projected onto a plane. A knot is a closed cyclic molecule that contains nodes that are unremovable (that is, they cannot be removed without breaking covalent bonds).

2.5. Characterization of Unusual DNA Motifs and Structures

The DNA branched junction is the first of several unusual DNA motifs that we will discuss in this article. It is important to mention the ways in which their structural features have been determined. Conventional physical techniques that are commonly used by chemists, for example NMR spectroscopy, have only recently yielded interesting data about these molecules,[29] and none of the species here has been crystallized yet. In general, we characterize a new motif first by means of non-denaturing gel electrophoresis, in order to establish whether the complex forms cleanly and with an appropriate molecular weight. On non-denaturing gels, hydrogen-bonded species migrate as a function of the molecular weight of the complex, as well as factors associated with the secondary structure such as the surface area. Bands appearing below the apparent monomer indicate instability,[22] and bands appearing above it indicate molecular strain, which can be relieved by making an oligomer.[30–32] Discrete bands indicate a closed complex (e.g., for a four-arm branched junction, molecule 1 forms hydrogen bonds to molecule 2, 2 to 3, 3 to 4, and 4 again to 1), but a smear of bands can indicate an open complex (1-2-3-4-1-2-3…), with no discrete closure. Stoichiometry is ascertained by titrating each strand against the others.[33] A Ferguson plot[34] is run in order to measure the friction constant of the complex, and to establish the dependence of mobility on gel concentration. Thermal denaturation profiles provide an estimate of the stability of the species.

The primary structural tool for analyzing these complexes is autofootprinting with $[Fe^{2+}EDTA]^{2-}$ (EDTA = ethylenediaminetetraacetate):[35] The pattern of chemical cleavage of the DNA by hydroxyl radicals generated by this reagent is monitored. The key to the analysis is that a radioactively labeled strand is complexed with its Watson–Crick linear duplex complement, and, in a separate experiment, with the other strands in the complex. Strands whose cleavage pattern is identical in both experiments are taken to be in a double-helical conformation; nucleotides that flank crossovers between helices exhibit a characteristic protection pattern. In addition to branched junctions, this type of analysis has been applied to antijunctions,[31, 32] mesojunctions,[31, 32] and double-crossover molecules.[30]

The more complex systems that are discussed here—a DNA cube, a DNA truncated octahedron, knots, and Borromean rings—are analyzed on denaturing gels. This is a system in which molecules of the same topology separate from each other as a function of their molecular weight; secondary structures are destroyed under denaturing conditions. Knotted molecules are compared with marker cyclic species in order to characterize their linking: The more tightly knotted a given strand is, the more rapidly it will migrate through a gel. All of these species contain restriction sites that are susceptible to restriction nucleases. In knots, lack of digestion is one of the demonstrations that a domain is in the Z conformation, and therefore contains positive nodes (see below). Catenated molecules are broken down to target catenanes or circles, for which markers can be prepared independently. Denaturing gel electrophoresis is very sensitive to topological differences: Two links differing only in their linking number will separate readily;[36] likewise, a linear catenane and a cyclic catenane of the same molecular weight are readily separated.[37]

3. Constructing DNA Objects and Arrays

3.1. Polyhedra and Solid-Support Synthesis

The flexibility of DNA branched junctions is a serious handicap if there is only a single complementary pair of sticky ends, as in Figure 6. However, a finite polygon or stick polyhedron can be made with a specific series of unique sticky ends. For example, to design a quadrilateral, a set of sticky ends {A, A′}, {B, B′}, {C, C′}, and {D, D′} will result in only of quadrilaterals or their multiples (octalaterals, dodecalaterals, and so on). As a practical matter, if the structure that has been designed is a favorable one, the cyclic tetramer will join to itself before larger cycles form in a solution of appropriate concentration. In our earliest attempt to produce a particular target molecule, we made a quadrilateral, which was purified readily from nontarget products.[38]

The first serious test of this system was the construction of a molecule whose helix axes have the connectivity of a cube;[37] its structure is illustrated in Figure 7. Each edge contains two turns of double-helical DNA; as with the square two-dimensional lattice, an integral number of turns per edge leads to cyclic strands. Each face corresponds to a cyclic strand linked twice to each of its four neighbors; hence, this object is a hexacatenane of DNA. Had we used an odd number of strands, the molecule would have been a tetracatenane corresponding to the projections down the body diagonals. To demonstrate synthesis, each edge contains a unique restriction site.

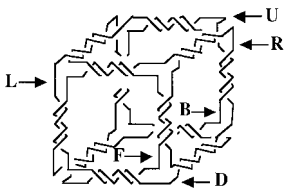

Figure 7. A DNA molecule whose helix axes have the connectivity of a cube. The molecule consists of six cyclic strands that have been catenated in this particular arrangement. They are labeled by the first letters of their positional designations: U: up; D: down; F: front; B: back; L: left; R: right. Each edge contains 20 nucleotide pairs of DNA, so we expect that their lengths will be about 68 Å. From model building, the axis-to-axis distance across a square face appears to be about 100 Å, with a volume (in a cubic configuration) of approximately 1760 nm^3 when the cube is folded as shown.

The synthesis is illustrated in Figure 8. Two squares were combined to form a ladderlike intermediate. One of the key lessons learned from this construction is that it is not possible to separate target molecules from by-products under non-denaturing conditions. Hence, we had to denature the complex, and then reconstitute it before a final round of ligation. The denatured intermediate was the linear triple catenane corresponding to the left (L), front (F), and right (R) faces of the cube. Among the most robust of our proofs of synthesis was the cleavage of the two edges contained in the L-F-R catenane to produce the linear triple catenane U-B-D, resulting from the up (U), back (B), and down (D) faces. Cleavage to release the U and D strands demonstrates that

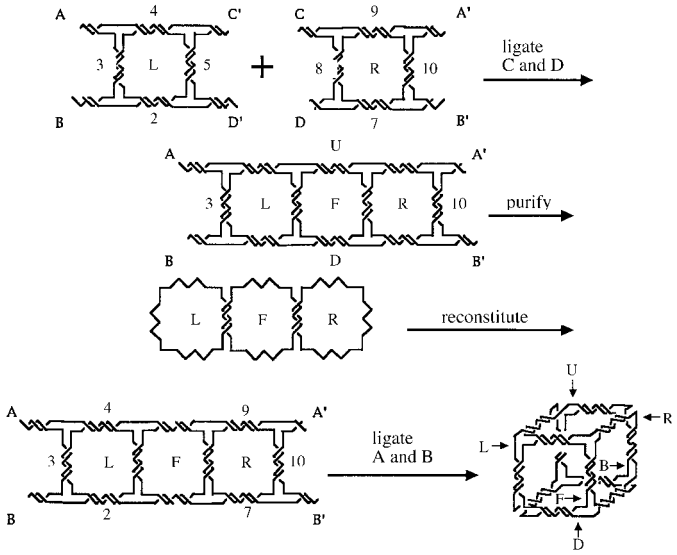

Figure 8. Synthesis of a DNA cube. The molecule is built from ten chemically synthesized strands, two 80-mers, and eight strands containing about 40 nucleotides. These are hybridized to form two quadrilaterals in the first step. Two ends (C and D) are ligated to form a beltlike molecule that must be denatured and reconstituted in order to purify it from side products. This molecule is then cyclized to form the cubelike molecule.

the target is the tetragonal prism, and not an octagonal or higher prism with $4N$ sides.

The synthesis of the cube was both a milestone and a disappointment. It was the first three-connected object synthesized from DNA, but the denaturation/reconstitution step clashed with the idea of forming specific edges through the specificity of DNA sticky ends. To restore the basic approach to this system we developed a solid-support methodology, patterned after the solid-support procedure used to synthesize oligonucleotides.[10]

The key advantage of the solid-support methodology[39] is that growing molecules can be isolated from one another on the support, so that the simplicity and specificity of sticky-ended ligation is not jeopardized by the presence of many copies of the same molecule in solution. In addition, the method permits the synthesis of individual edges at a time; control derives from the restriction of hairpin loops forming each side of the new edge. This corrects one of the major problems encountered in the cube construction, where a double ligation in the first step led to unwanted by-products. The strategy permits the separate execution of steps involving additions and cyclizations, which are optimized under different conditions. Each cycle of the procedure creates an object that is covalently closed and topologically bonded to itself, enabling some purification to take place on the support. As with other syntheses using solid supports, this methodology permits convenient removal of reagents and catalysts from the growing product.

Syntheses are designed so that the pair of restrictions that create a sticky end have different flanking sequences; hence the ultimate ligated edge contains a site for neither enzyme, and cannot be digested by it. For example, a typical pair of sites would be *Xho* I (C|**TCGA**G) and *Sal* I (G|**TCGA**C) (sticky ends in boldface); the ultimate sequence would be CTCGAC (paired with GTCGAG), immune to both enzymes that might be present in solution as contaminants, but sensitive to *Taq* I (TCGA), which is important for analytical purposes. It is possible to use symmetric sites for intramolecular ligations on the solid support, but it is unwise to use them in solution, because one cannot control whether the products will be intramolecular or intermolecular. In principle, the best enzymes to use for intermolecular ligations are those that produce asymmetric sticky ends. These can be produced by restriction enzymes that cleave at interrupted sites (e.g., *Bgl* I (GCCn**NNN**|nGGC)) or those that cut at positions distant from their recognition sequences (e.g., *Bbs* I, which leaves 5′ **NNNN**nnGTCTTC as a sticky end). Here, **N** represents any nucleotide that will be exposed as part of a sticky end, and n a nucleotide whose position is part of the enzyme's specificity, but its identity is not. However, as a practical matter, these enzymes are often less effective and more expensive than the more common enzymes with symmetric cleaving sequences.

Figure 9 illustrates a square synthesis: A three-arm branched junction with arms ending in hairpins is attached to the solid support, and one of its arms is restricted to expose a sticky end. This molecule is ligated to a second three-arm

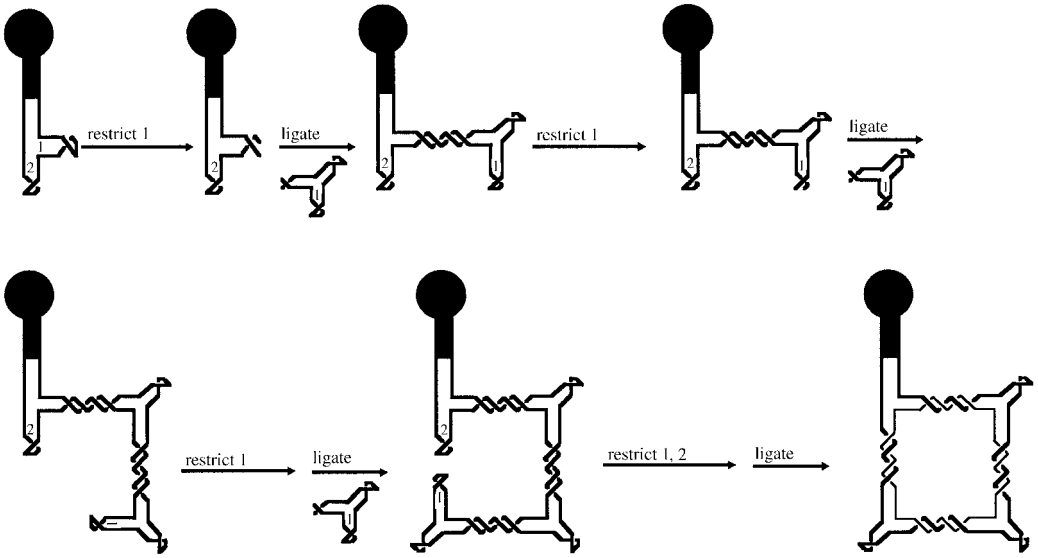

Figure 9. Synthesis of a quadrilateral. Beginning with the support containing a closed junction, alternate cycles of restriction and ligation are performed, always at position 1. Selection of the target product (triangle, quadrilateral, pentalateral…) is determined by the point at which one chooses to restrict at position 2, exposing a sticky end complementary to that exposed by restriction at position 1. This action corresponds to a strand switch (eliminating a zero node; see Figure 14). This is emphasized by the lines of different thickness with which the "square" catenane is drawn.

junction, consisting of two hairpins and an arm ending in the complementary sticky end. This process is repeated until the target precursor to a square is obtained. This is followed by a final round of double restriction and an intramolecular cyclization to produce the squarelike molecule; this species will be removed from the support and annealed shut by the addition of a hairpin.

To challenge and refine this methodology, we have constructed a truncated octahedron from DNA.[40] This Archimedean solid contains six squares flanking its fourfold symmetry axes, and eight hexagons that surround its threefold symmetry axes. As with the cube, there are two turns of DNA per edge, so each of the 14 faces corresponds to a single cyclic strand, and the final object is a [14]catenane. Each vertex of a truncated octahedron is bonded to three others (three-connected), but the molecule has been constructed from four-arm junctions; consequently, each vertex is associated with another arm that could be used to join the polyhedra, although this has not been done. The extra arms are hairpins extending from the strands that correspond to the square faces. The entire molecule contains 2550 nucleotides and has a molecular weight of about 790 kDa.

In this construction, the objects added to the support are squares and square groupings (Figure 10). The target has been made by carrying out two intermolecular additions to a square attached to the support. In the first addition, a tetrasquare complex is added, and in the second addition, the final square is added. The reason for adding the tetrasquare complex is that some restriction enzymes were found to be inefficient in the presence of the solid support. The structure on the lower left of Figure 10 is a heptacatenane complex of six squares. The square strands are already intact in this construct, and the hexagons all derive from the outer strand. The hexagons result from successive intramolecular closures of the sticky ends associated with the restriction enzyme site pairs S1-S1′ to S7-S7′. The final step involves releasing the structure from the support and annealing it with a hairpin. Proof is demonstrated in two stages: First, by showing that all six cyclic strands corresponding to the squares are in the heptacatenane, and second, by digesting the final product to the tetracatenanes that flank the squares. Once the ultimate goals of this program have been realized, it will be possible to characterize such targets by physical methods, particularly X-ray crystallography. However, characterization today is reminiscent of

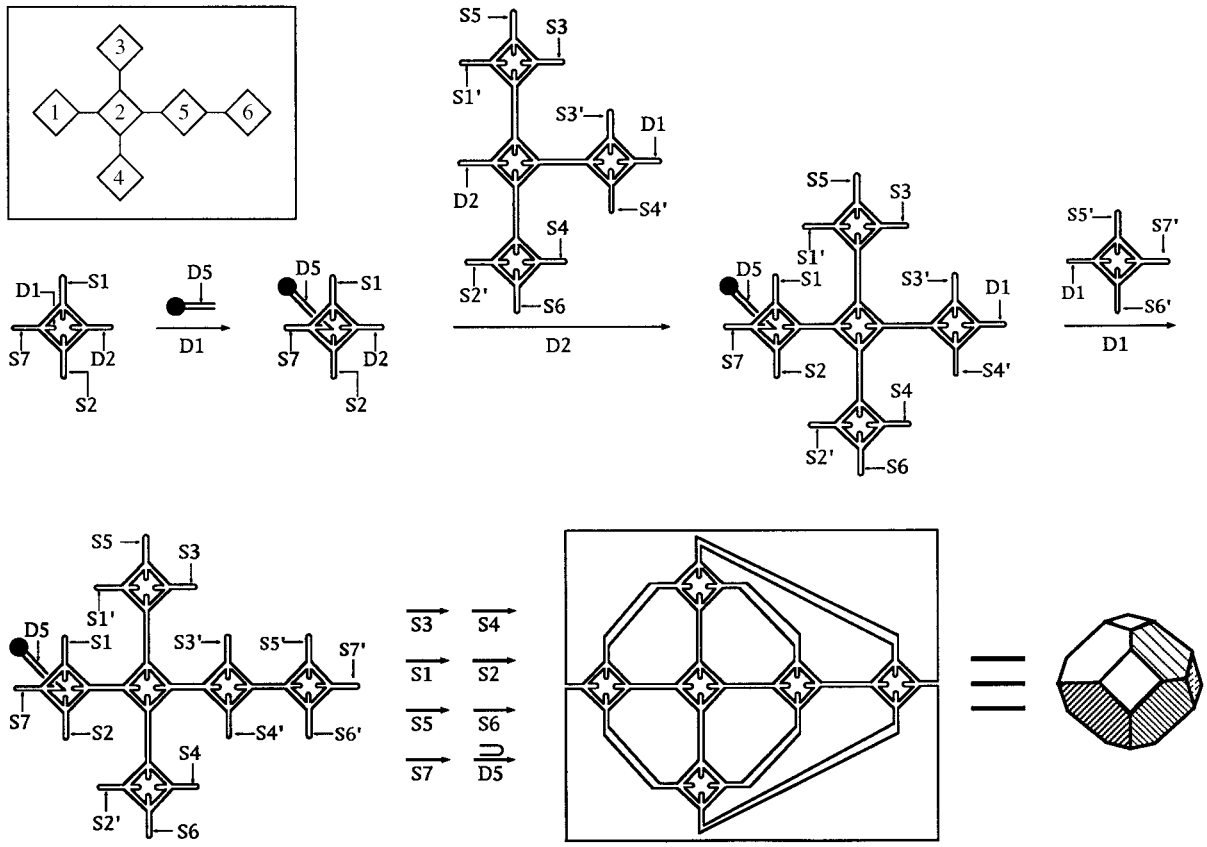

Figure 10. Synthesis of a truncated cctahedron on a solid support. The boxed diagram in the upper left corner indicates the numbering of the individual squares. Each square in the rest of the diagram is shown with its restriction sites indicated. Symmetrically cleaving restriction sites are indicated pairwise with S and S′; restriction sites that are cut distally by type II restriction enzymes are named D; restriction sites on the exocyclic arms are not indicated. The arms that will eventually be combined to form edges of the object are drawn on the outside of each square, and the exocyclic arms are drawn on the inside of the square. A reaction is indicated by an arrow above the name of a restriction site: This means that the restriction enzyme (or enzyme pair for those labeled S) is added, the protecting hairpins are removed, and then the two sticky ends are ligated together. The product is shown in two forms. On the left, the S1 – S6 closures are shown as triple edges to emphasize their origins; the two strands of the edge formed by the S7 closure are separated to maintain the symmetry of the picture. On the right, a rotated front view of a polyhedral representation of a truncated octahedron is shown without the exocyclic arms; the symmetry of the ideal object is evident from this view.

chemical proof before physical techniques came to the fore: We break molecules down to predictable fragments for which it is possible to make independent electrophoretic markers. As noted above, characterization, like synthetic control, is topological, rather than geometrical.

3.2. DNA Double-Crossover Molecules

Flexibility is the key shortcoming of DNA branched junctions as components for DNA nanostructure. We have demonstrated above that the control of topology in this system is strong, but this leads only to limited structural control. The variation of sticky ends can produce particular closed species in flexible systems, such as the cube and truncated octahedron. From the standpoint of sequence, these are low-symmetry structures, because all of the sticky ends are unique. However, the construction of periodic matter entails high-symmetry components, because the opposite faces of each unit cell are complementary. To address this problem, we have sought new DNA motifs to serve as rigid components. Initially, we were drawn to bulged branch junctions because of reports of their structural rigidity, which were borne out in preliminary experiments.[28] However, more rigorous testing showed that this motif was not sufficiently stiff for the purposes of DNA nanotechnology.[41] Fortunately, we have come across a system that appears to be much more rigid, the DNA double-crossover molecule (DX).[30, 42] This is a DNA structure that is an intermediate in the genetic process of meiosis.[43] It consists of two four-arm branches joined at two adjacent arms.

There are five isomers of DX molecules (Figure 11). Three of these isomers contain parallel (P) double-helical domains; this means that the dyad axis of backbone symmetry in DPE, DPOW, and DPON lies within the plane of the paper, parallel to the helix axes of the domains. The two other isomers DAE and DAO contain antiparallel helices, so that the dyad axis is perpendicular to the helix axes; in DAE it is perpendicular to the plane of the page and broken by the nick in the central strand, and in DAO it is horizontal within the plane of the page. The third descriptor of these isomers, E or O, signifies that the crossovers are separated by an even (E) or odd (O) number of double-helical half-turns. The DPO isomers are differentiated by whether the extra half-turn is a major groove (W for wide) separation or a minor groove (N for narrow) separation. Note that between the crossovers the strands of the two domains approach each other closely. There is electrostatic repulsion between these strands, because they are both negatively charged. Consequently, parallel isomers are not stable when the separations are very short, and they will not be discussed further here. Nevertheless, they have been used in analytical studies of recombination components,[36, 44] as have antiparallel DX molecules.[44, 45]

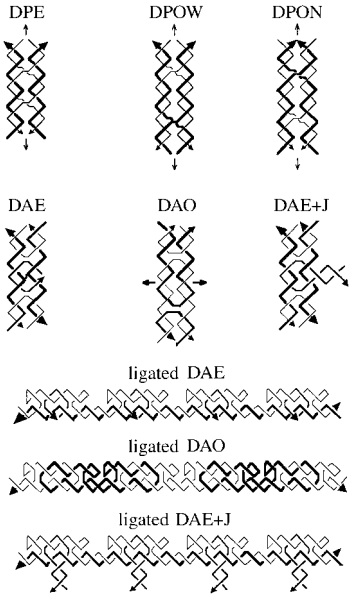

Figure 11. Double-crossover molecules and their ligation products. Arrowheads indicate 3′ ends of strands. Strands drawn with the same thickness are related by the vertical dyad axis indicated in the plane of the paper. In the case of DAO, strands of opposite thickness are related by symmetry. See text for further details.

The structure DAE + J on the right side of the middle row of Figure 11 is a variation on the DAE molecule. This molecule is created by replacing the nick of a conventional DAE (hard to seal when the separation is a single turn) with a bulged junction. It is also possible to produce molecules with multiple arms extending from the central portion of a double-crossover molecule: Winfree has constructed DAO + 2J molecules, and we have constructed DAE + 2J molecules;[48] in both cases, the extra helices are directed normal to the plane containing the helix axes.

The bottom portion of Figure 11 illustrates the topological consequences of oligomerizing one domain of these molecules. Oligomerization of DAE or DAE + J produces a reporter strand. However, oligomerization of DAO leads to a polycatenane. Owing to the difficulty of characterizing polycatenanes, we have explored the structural rigidities only of DAE and DAE + J.[42] In both cases, long reporter strands result; only minimal cyclization is observed with DAE + J, and there is no evidence for cyclization of DAE. The DAO molecules form closed structures more readily than DAE molecules, because they do not contain the cyclic central strand that is usually left unsealed in DAE molecules; we expect that DAO molecules are likely to be stiff, because they lack this nicked strand. Consequently, we conclude that antiparallel DX molecules represent a motif offering the needed rigidity in DNA construction.

3.3. DNA Arrays

The minimal requirements for a component to be used in the design of periodic matter are 1) predictable intermolecular interactions, 2) predictable local product structures, and 3) structural integrity.[28] Condition 1 is key, because the local affinities must be established under conditions of crystallization; condition 2 is needed to know how to design the lattice; and condition 3 is required if the system is to be translationally symmetric. In principle, one could imagine using many different sticky ends to create a pseudocrystal (same backbone, different sequences) held together with the same

specificity as the cube or the truncated octahedron. However, the preparation of those components would be prohibitively expensive; a conventional crystal is the cheapest way to assemble macroscopic materials whose structure is precise. We have seen above that the specificity of DNA is simple to design, and that the local structure at a sticky-ended overhang is B-DNA.[8] A known local structure is a subtle but key feature for the assembly of any specific geometrical species; for example, the structure of a sticky-ended cohesive join would be different if the overhangs assumed an exotic structure, such as parallel DNA.[46] In the last section, we introduced a DNA motif that satisfies the final criterion, molecular rigidity. Thus, in DNA, we have all the features necessary to form periodic matter.

How can we incorporate DNA antiparallel DX molecules into the self-assembly context described above? At least two routes seem promising. One way is to assemble arrays of DX molecules themselves. Winfree has identified four different topologies of antiparallel DX arrays.[47] These are illustrated in Figure 12. The arrays are made up of DAE or DAO

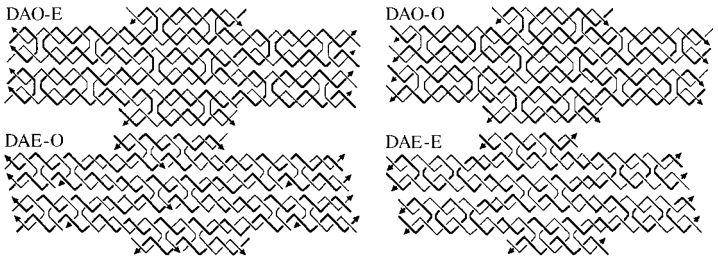

Figure 12. Topological structure of two-dimensional antiparallel DX arrays. Strand polarities are shown by the arrowheads on their 3′ ends. The strands are drawn with different thicknesses for clarity. See text for further details.

molecules, separated by even (DAE-E and DAO-E) or odd numbers (DAE-O and DAO-O) of double-helical half-turns. The topology of DAE-E is molecular chain mail. DAE-O produces two reporter strands in both the horizontal (straight) and vertical directions (zigzag). The DAO arrays lead to zigzag reporter strands in the vertical direction only. In our laboratory we have made DAE-O arrays, demonstrated by reporter strands, streptavidin gold labeling in the AFM, and DAE + 2J components; the 2J hairpins act as a topographic label. Winfree et al. have demonstrated[48] the assembly of DAO-E arrays by reporter strands and by means of DAO + 2J topographic labels in the AFM. We have shown that changing components leads to predictable alterations of AFM images.[48]

A second route to incorporating DX molecules into periodic matter is to have them serve as the edges of triangularly based species. This means using triangles in two dimensions and deltahedra (polyhedra whose faces are all triangles) in three dimensions. Triangles and convex deltahedra are rigid species, regardless of the flexibility of the angles between their edges.[49] Figure 13a illustrates a molecule we have made recently, a triangle with two sides composed of DAE molecules. Ligation in one dimension leads to a reporter strand that shows no evidence of cyclization on gels or by

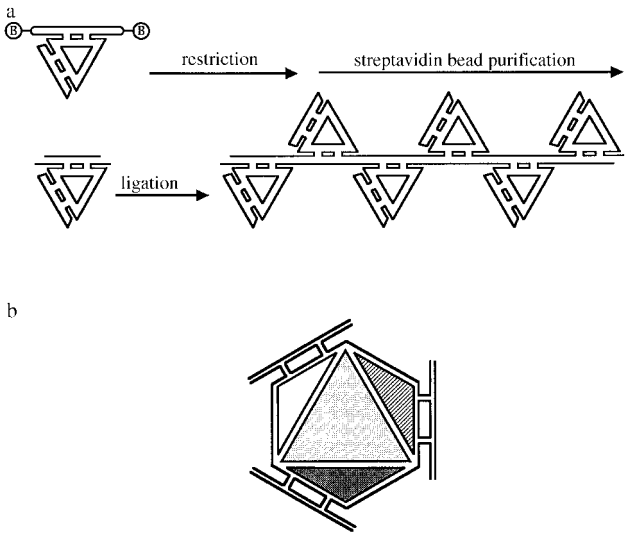

Figure 13. Structures containing double crossover. a) DX molecules incorporated in the edges of a triangle. Two edges of a triangle contain DAE molecules. Oligomerization will take place in the edge containing biotins (B) following restriction of those hairpins. b) An octahedron containing DX molecules. The top four faces of an octahedron are shown. Three edges that span a three-dimensional space contain DX molecules. The system would be ligated through the extra domains of the DX molecules.

AFM examination. Figure 13b shows a possible means of extending this system, with different components, to three dimensions: Three edges of an octahedron are shown to incorporate DX molecules. Despite their appearance in the drawing, these edges are not coplanar, but span three-dimensional space; sticky-ended cohesion along the outer domains would lead to a rhombohedral lattice. Note that the vertices of the octahedron are four-arm branched junctions, because the octahedron is a four-connected object. In a future scenario of polyhedral lattice construction in solution,[41] one can imagine building topologically closed complex objects (such as the octahedron) on solid supports, releasing them into solution, and annealing them to closure. They could then be purified under the denaturing conditions that work best for this purpose. Following purification, they could be restricted in solution, have their hairpins removed by streptavidin treatment, and be allowed to assemble.

4. Topological Construction

4.1. DNA and RNA Knots

The importance of topology in chemistry has been recognized at least since the work of Wasserman and Frisch,[50] and many investigators have made valuable experimental contributions in this area.[51] The investigators who have written the articles in reference [51] have synthesized a plethora of topological targets from small molecules, including a variety of knots and catenanes. All of their achievements required a great deal of chemical skill; by contrast, the production of deliberate single-stranded topological targets from DNA is relatively simple and straightforward.

There are three reasons why work on DNA geometrical objects has led to the construction of DNA (and RNA) topological targets: 1) Geometrical DNA objects are catenanes, and related closely to knots; 2) the nucleic acid double-helical half-turn is a ready-made unit tangle, the basic constituent of all knotted and catenated species; and 3) knots offer a potential route to the biological production of DNA polyhedra. Each of these points requires detailed explanation.

Figure 14a illustrates a transformation that interconverts knots and catenanes. At the left is a knot with five nodes, and the polarity of the strand is shown by the arrowhead (3′ end). In the middle molecule, one node has been destroyed. To see

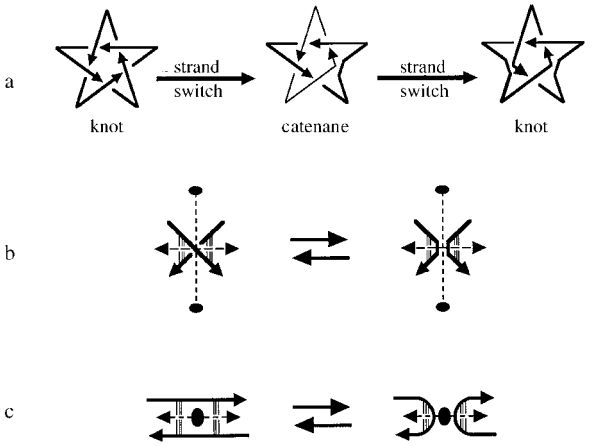

Figure 14. Zero-node operations. See text for details.

how this is done, divide each strand into a substrand before the node and a second substrand after the node, but maintain the polarity of each substrand. The node has been destroyed by switching the attachments of the substrands while maintaining their polarities. When this operation is performed, the knot is converted into a catenane. The drawing on the right indicates that the same operation applied to the catenane converts it into a knot. The operation shown is sometimes termed converting a positive or negative node into a zero node.[52]

It is useful to put this operation into the context of DNA.[53] This is shown in Figure 14b for a single node. The horizontal double-headed dashed line represents the DNA helix axis, and the vertical dashed line terminating in ellipses represents the DNA backbone dyad axis perpendicular to the helix axis. The strands are shown as arrows, which represent their polarities, and six nucleotide pairs connect the strands. Zero-node formation destroys the node, creating the structure on the right. Figure 14c illustrates this same operation in the same system, but the DNA is rotated by 90° about the helix axis, so that we are looking down the backbone dyad axis. In this view it is clear that the operation cleaves the two strands and creates two opposing hairpins. Note that in transforming this operation from topology to chemistry, we have replaced the right arrow with a pair of arrows indicating a reaction. The right arrow indicates the analytical operation devised by mathematicians to analyze knotted topology.[52] However, the arrow pointing left indicates a synthetic operation discussed in assembling DNA objects. Comparison of Figure 14c with the final step in Figure 9 shows that combining two intramolecular sticky ends, by the restriction/ligation operation, converts a knot (the trivial knot here, the circle) into a catenane; the two strands in Figure 9 are drawn with lines of different thickness to emphasize this point.

The fundamental unit of linking and knotting topology is the node or unit tangle.[24] Every knot or link is characterized by the number and relative positions of its nodes. Figure 15a illustrates that the double-helical half-turn can act as a unit tangle, thereby making the transition from topology to nucleic acid chemistry. A trefoil knot has been drawn with thick lines, and arrowheads indicating its trajectory. A half-turn of base paris is drawn between antiparallel strands, and the helix axes and dyad axes are shown. A dashed square has been drawn around each of its nodes, so that the strands of the knot act as its diagonals. The diagonals divide the square into four sections, two between parallel strands and two between antiparallel strands. The transition from topology to chemistry is made by drawing the six base pairs that flank a double-helical half-turn between the antiparallel strands. The rest of the knot can be made of unstructured oligodeoxythymidine (oligo-dT) units. Figure 15b indicates that nodes may possess either of two opposite chiralities. Negative (−) nodes are produced by right-handed B-DNA, the conventional structure of DNA. However, it is possible to produce positive (+) nodes by using left-handed Z-DNA (which has a zigzag nature).[54] Z-DNA is not geometrically enantiomorphic to B-DNA, but it is enantiomorphic in the topological sense.

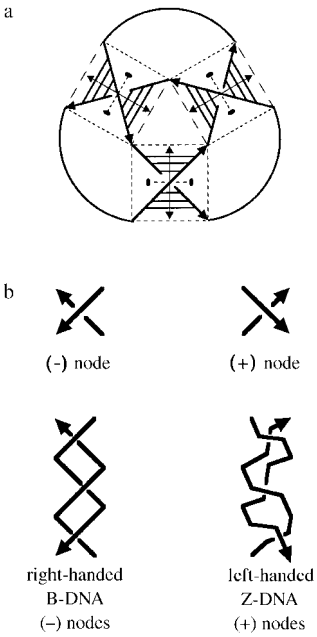

Figure 15. DNA topological methodology. The relationship between a half-turn and a node as well as the signs of the nodes is shown. See text for details.

By using the relationship between double-helical half-turns and nodes, we have constructed a variety of topological species. In each case, a complete DNA strand is synthesized, but the final ligation that circularizes the molecule is done enzymatically. We have made trefoil knots from two motifs of B-DNA,[55, 56] a figure-eight knot from a strand containing two negative nodes (B-DNA) and two positive nodes (Z-DNA),[57] and a trefoil knot containing positive nodes.[58] The figure-eight knot is a topological rubber glove,[59] that is, a structure whose molecular graph can be deformed to its mirror image, but cannot be deformed to a symmetry presentation.

Two conditions must be fulfilled for a segment of DNA to convert from B-DNA into Z-DNA: 1) The sequence must be

amenable to forming Z-DNA; and 2) the molecule must be in solution conditions that promote the B →Z transition, such as high ionic strength or in the presence of special cationic effectors, such as $[Co(NH_3)_6]^{3+}$.[54] Thus, the same strand can be used to create a variety of knots, depending on the conditions under which the final ligation takes place. For example, we have made a single strand adopt four different topologies by controlling ligation conditions, as shown in Figure 16. The regions X and X′ are complementary, as are the regions Y and Y′. Both regions can pair to form a single turn of DNA, and both regions can undergo the B →Z transition, although the Y-Y′ double helix has a greater propensity to form Z-DNA than the X-X′ double helix. Ligation at very low ionic strength results in only a circle. Increasing the ionic strength leads to the formation of a trefoil knot containing negative nodes, as both domains form B-DNA. Greater ionic strength converts the Y-Y′ domain into Z-DNA, producing a figure-eight knot. The addition of $[Co(NH_3)_6]Cl_3$ converts the X-X′ domain into Z-DNA as well, which generates the positive trefoil knot.

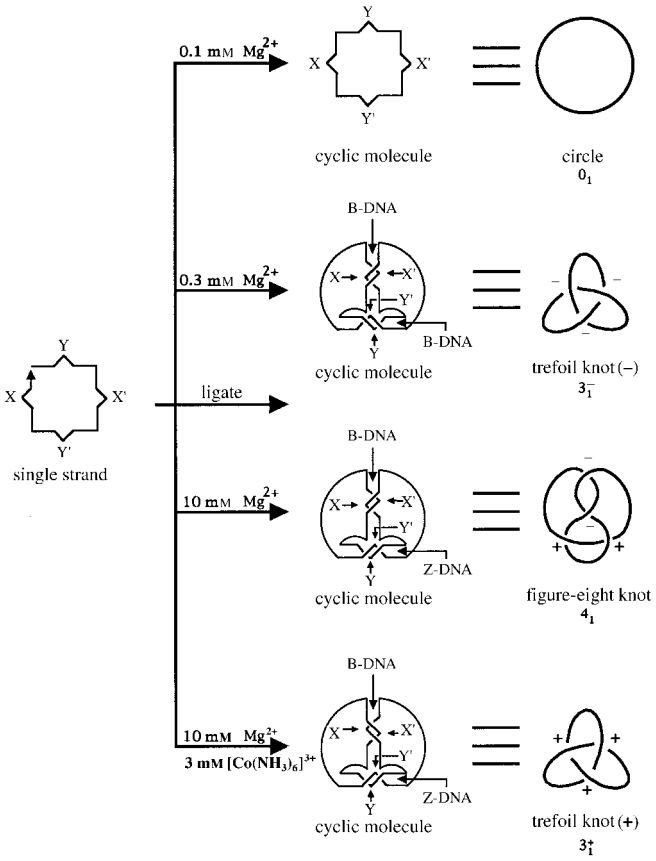

Figure 16. Synthesis of four different topologies from a single DNA strand. On the left is shown the molecule from which the target products are produced. The four pairing regions, X and its complement X′ and Y and its complement Y′, are indicated by the bulges from the square. The 3′ end of the molecule is denoted by the arrowhead. The four independent conditions used to generate the target products are shown to the right of the basic structure. The pairing and helical handedness expected in each case is shown to the right of these conditions, and the molecular topology of the products is shown on the far right of the figure. For an explanation of the topological sybols K_i, see Section 4.3.

We have shown that each of the species in Figure 16 can be interconverted through catalysis by DNA topoisomerase I or III from *E. coli*.[60] It is possible to ligate a knot under a certain set of conditions, and then to change the solution conditions to those favoring a different topological species. Even though a new species is favored, the covalent closure of the molecule prevents it from assuming that topology; for example, a figure-eight knot (containing a Z-DNA domain) removed from Z-promoting conditions cannot convert into a trefoil knot without the breakage of bonds. However, single-stranded (Type I) DNA topoisomerases can catalyze this conversion, because they facilitate strand-passage operations.

The efficient catalysis of strand passage in these knots by DNA topoisomerases suggested that knots might be a useful substrate to discover another biological activity, that of an RNA topoisomerase. In particular, we sought to determine whether the suggested activity of DNA topoisomerase III from *E. coli* as an RNA topoisomerase[61] could be established. We constructed a single-stranded RNA knot, and a circle of the same sequence. DNA topoisomerase III from *E. coli* was able to catalyze the interconversion of these species;[15] in addition, the enzyme catalyzed the catenation of a small amount of RNA. The instability of RNA, and the lack of effective modifying enzymes (no restriction enzymes, no exonucleases that lacked endonuclease contamination, and inefficient ligation by DNA ligases), converted a relatively straightforward experiment into a difficult technical project. Although related only indirectly to nucleic acid nanotechnology, the difficulties encountered in the discovery of the RNA topoisomerase have had a strong impact, reinforcing our inclination to work with standard DNA backbones.

DNA knots differ somewhat from DNA polyhedra: Polyhedra contain no unpaired nucleotides, but there are long single-stranded linker regions between pairing domains in knots. One has little control over the structures that can be assumed by single-stranded DNA. A possible solution to this problem is the temporary addition of single-stranded DNA complementary to the linker regions. Thus, their interactions are controlled during the ligation process; whereas these molecules are not topologically bonded to the target molecule, they can be removed readily upon purification of the product. This idea is illustrated for a catenane in Figure 17. We have shown that this procedure is quite effective when applied to catenanes of arbitrary sequence.[36] Ironically, even though topological protection was devised with knots in mind, it has proved singularly unsuccessful in that application. Indeed, attempts to apply topological protection have often resulted in larger amounts of undesired products.[56] We ascribe this failure to the oligo-dT sequences in the linkers; the complementary sequence, oligo-dA, is capable of forming triple helices, involving two linkers simultaneously, and leading to their linkage.

We suggested above that knots could offer a route to the biological production of DNA polyhedra. How could this be done? Figure 18a shows that replication of a stable branch by DNA polymerases is not possible: The heteroduplex region present in the first generation would be eliminated in the second generation if the DNA were replicated either intracellularly or by PCR. However, it may be possible to produce

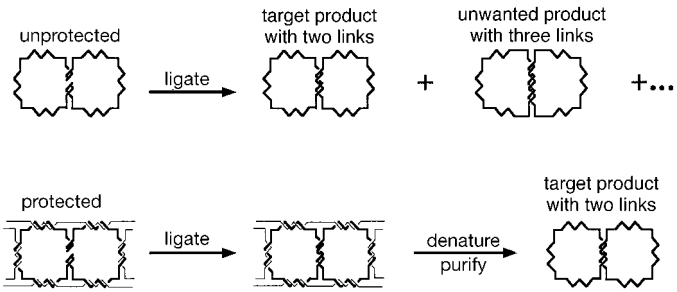

Figure 17. Topological protection of catenanes. This figure illustrates two catenation reactions: In the top reaction the two strands are ligated together in the absence of topological protecting groups, and in the bottom reaction topological protecting groups are present during ligation. The topological protecting groups are drawn as much thinner lines than the molecules to be linked together. In both cases, the final catenane has two turns of Watson – Crick complementarity, seen in the central duplex, where they are hybridized together. In the top reaction, the target doubly linked catenane is shown as a product, but another catenane is also drawn which contains an adventitious link. In the lower reaction, all the nucleotides of noncomplementary DNA were paired with their Watson – Crick complements, and were removed upon denaturation.

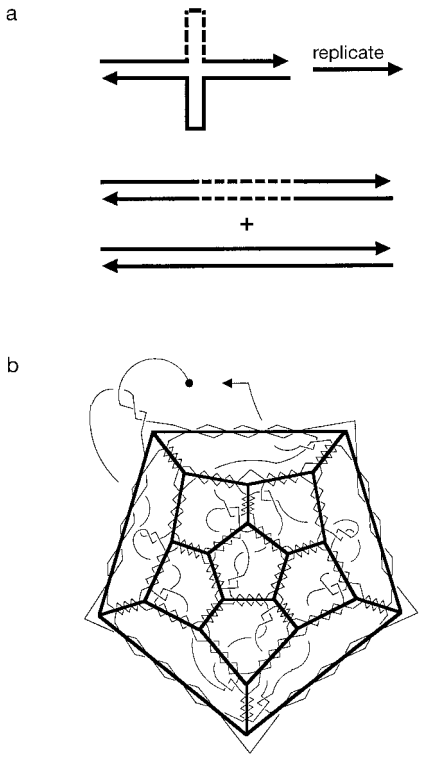

Figure 18. Cloning of branches. See text for details.

the molecules as a single long strand (Figure 18b).[62] A Schlegel diagram of a pentagonal dodecahedron is shown. Each edge is decorated with two turns of DNA; thus, each face corresponds to a cyclic strand. Each cycle has had an exocyclic arm added to it, and these exocyclic arms have been connected to form a single long strand. Biologically based production of this object might be accomplished by producing the strand, allowing it to fold (probably in the presence of Type I DNA topoisomerases), and then restricting the arms to remove the connecting strands. This protocol could lead to a polyhedron ready to connect to others through the sticky ends generated by restriction. Clearly the folding of the strand would need to be programmed very carefully, and then controlled closely, possibly by temperature.

4.2. DNA Borromean Rings

To challenge the level of topological control available with nucleic acids, we have used this system to construct Borromean rings.[63] These are a rich family of topological structures,[64] whose simplest member is a three-ring link (Figure 19, upper left) on the crest of the Borromeo family,

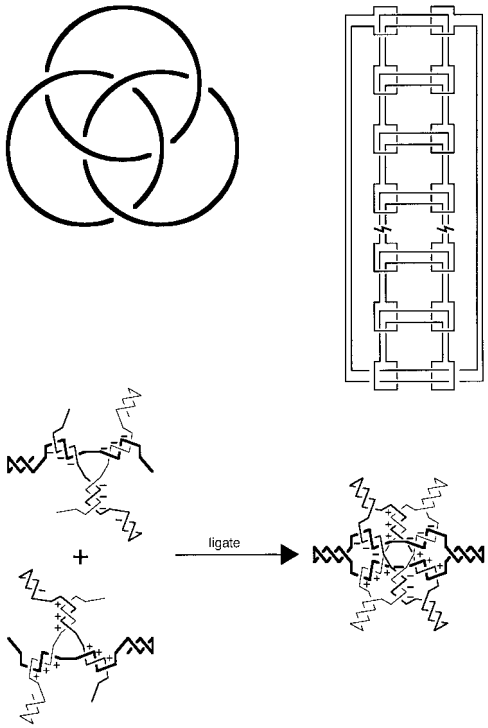

Figure 19. Borromean rings. Traditional Borromean rings are shown in the upper left corner; no two rings are linked. The diagram in the upper right illustrates the fact that an arbitrarily large number of rings can be linked in a Borromean arrangement, so that cleavage of any one of them results in the unlinking of the entire complex. The drawing at the bottom illustrates the synthesis of Borromean rings from DNA. A B-DNA branched junction (top) and a Z-DNA junction (bottom) are shown, each with 1.5 turns per arm; the signs of nodes are noted. The three strands are drawn with lines of different thickness. At the ends of each strand are partial hairpins (one almost complete, one that supplies the missing portion to another incomplete hairpin). When ligated under conditions that promote formation of the Z-helix, the Borromean arrangement on the right is formed.

prominent in the Italian Renaissance. The special property of Borromean rings is that cleavage of any member of the link results in separation of the other members of the link. This is evident in Figure 19, because no pair of rings are linked. The upper right portion of Figure 19 shows that there is no limit to the number of rings that can be joined while retaining the Borromean property.

In the three-ring link, the signs of the three outer nodes are opposite to those of the three inner nodes. Each of these nodes could be generated by utilizing a half-turn of double-

helical DNA. To simplify the construction, we replaced each of the half-turns of DNA with three half-turns of DNA, because that is a more stable structure. This is a topological structure, so it is possible to wrap it around a sphere. When this is done, the inner three helices correspond to a three-arm B-DNA branched junction flanking the north pole, and the outer three helices correspond to a three-arm Z-DNA branched junction flanking the south pole. We included hairpins on the "equator" to provide sites for ligation (Figure 19, bottom). These hairpins also contained restriction sites, so that the circles could be individually cleaved. Although not indicated in Figure 19, the hairpins were of slightly different lengths, so that the three circles could be separated conveniently on denaturing gels. Proof of synthesis consisted of cleaving each of the circles individually and showing that the products consisted only of the other circles and did not include any dicatenanes.

4.3. DNA Antijunctions and Mesojunctions

Working with knots has led to a generalization of the branched junction. If one designs a knot by the principle described in the last section, the arrangement of nodes leads to a series of substructures. For example, knot 9_{49} is shown in Figure 20a with every node written as a double-helical half-turn between antiparallel strands. (In the notation K_i for a knot, K refers to the number of nodes in a minimal plane representation of the knot, and i differentiates distinct knots with identical K values.[52]) The polarity of the knot is indicated by the direction of the arrow passing along it. Some of the enclosed regions contain other symbols that describe ways in which the double helices could be condensed: A solid triangle indicates that it is flanked by three double helices arranged like the arms of a three-arm branched junction, because their helix axes point towards the interior of the region. To its right are two helices connected by a curved double arrow, because these two half-turns could be condensed into a full turn of duplex DNA. To the left of the solid triangle is another area containing an unfilled square. Four half-turns flank this region, but they are not related like the arms of a branched junction, because their helix axes are circumferential, rather than pointing to its center. This is a new topology, termed an "antijunction".

The relationship between a junction and an antijunction is shown more clearly in Figure 20b. Here, the conventional four-arm branched junction is designated as 4_4 and the four-arm antijunction as 4_0, where the subscript indicates the number of arms pointing radially. The helix axes of the branched junction point towards the middle of the square, whereas the local dyad axes of the antijunction point towards the center. It is possible to switch the orientations of pairs of double helices without creating topological ambiguities, resulting in "mesojunctions". The two four-arm mesojunctions are designated as $^1\!4_2$ and $^2\!4_2$, where the subscript again indicates the number of radial helix axes, but the superscript is a serial number to distinguish the two species. Figure 20b also illustrates a three-arm branched junction (3_3) and the only possible three-arm mesojunction (3_1).

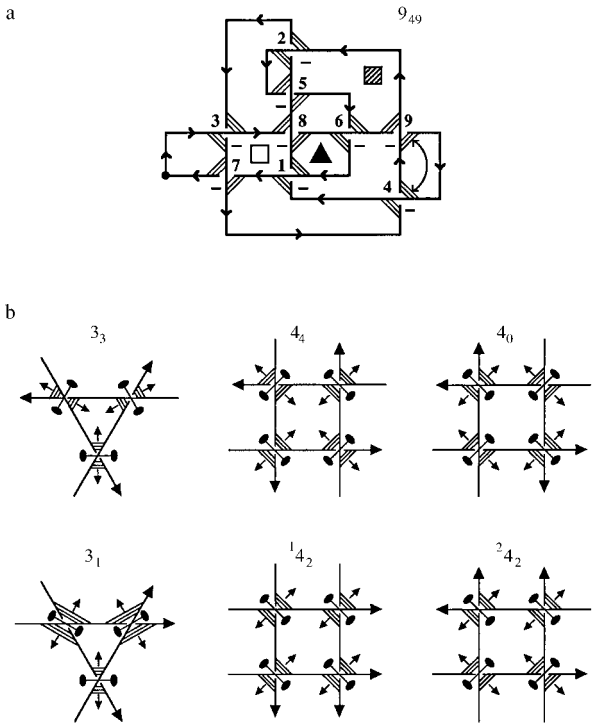

Figure 20. Antijunctions and mesojunctions. a) A 9_{49} knot drawn in the context of DNA. See text for details. b) Schematic drawings of three- and four-strand junctions, antijunctions, and mesojunctions. They are shown as helical arrangements that can flank a triangle or a square. Each polygon is formed from strands of DNA that extend beyond the vertices in each direction. The arrowheads indicate the 3′ ends of the strands. The vertices correspond to the nodes formed by a half-turn of double-helical DNA. Base pairs are represented by lines between antiparallel strands. Thus, the notation is similar to that used in (a), in that each set of six base pairs represents a half-turn of double-helical DNA. However, the half-turns are decorated more extensively: Thin arrows perpendicular to the base pairs, and pointing away from them, represent the axis of each helical half-turn; and the lines perpendicular to the helix axes terminating in ellipses represent the central dyad axes of the helical half-turns. The complexes 3_3 and 4_4 correspond to conventional branched junctions, whose helix axes (the arrows normal to the base pairs) point to the center of the triangle or the square. The complex 4_0 is a four-strand antijunction, whose helix axes are circumferential about the square, but whose dyad axes point towards the center of the square. The complexes on the bottom row are mesojunctions which contain a mix of the two orientations of helix axes.

We have constructed the antijunction and mesojunctions shown in Figure 20b. The stability of these species is a function of the number of half-turns in circumferential domains. We have attempted to construct these species with two and with three half-turns in each domain.[31, 32] These are not tractable species, and are much less stable than conventional branched junctions. Unless assembled at very low concentrations, mesojunctions form discrete closed oligomers of the target complex. Antijunctions often form open complexes (e.g., complexes like 1-2-3-4-1-2-3.., where the numbers refer to different molecules of a particular numbered strand); in contrast to closed complexes, there is no well-defined cyclic species found here.

Examination of antijunction and mesojunction molecules reinforces the notion that stacking may be the key element to consider in the design of DNA secondary structure. Structures that maximize base stacking appear to be favored in these systems, if they are compatible with the twists of the species

involved. Considering the origin of antijunctions and mesojunctions in nucleic acid knots, it is not surprising that they are related closely to pseudoknots seen in cellular RNA structures. In general, the intractability of antijunctions and mesojunctions militates for avoiding them in topological constructions. For example, it was possible to design Borromean rings as the sum of either two 3_1 mesojunctions or two 3_3 conventional junctions; the selection of the two conventional branched junctions was not a difficult decision to make.

5. Nanomechanical Devices

It is desirable to combine architectural properties of DNA with its dynamic properties in order to produce nanomechanical devices. There are two prominent transitions that can be utilized for this purpose. The first of these is branch migration[17] (Figure 21 a). In general, this transition can go in either direction; in a small system, the four-arm junction will resolve to two duplex molecules. However, the transition

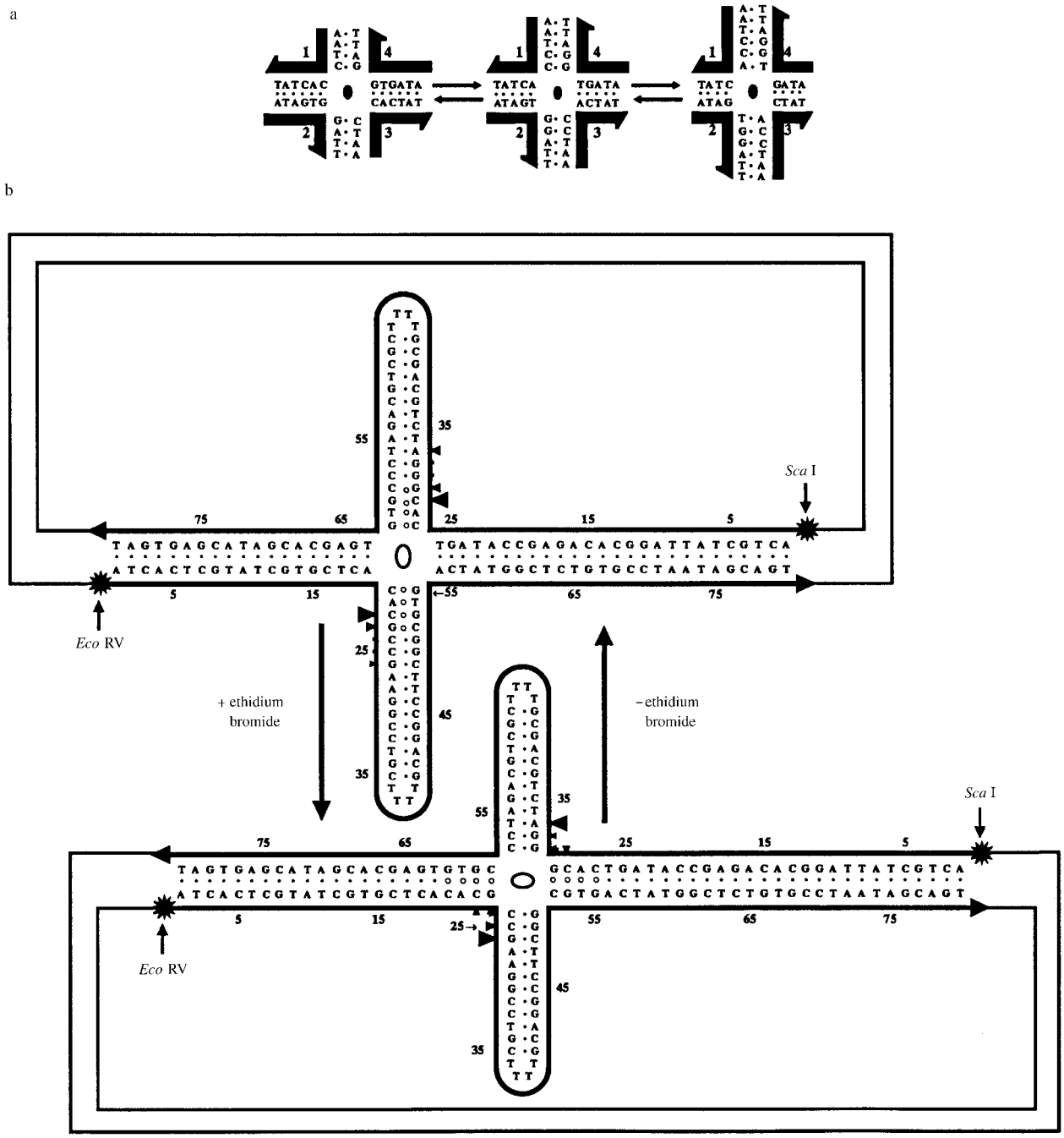

Figure 21. Control of branch migration. a) Branch migration. A four-arm branched junction is shown with twofold sequence symmetry about its branch point. The steps from left to right lead to the movement of base pairs from the horizontal arms to the vertical arms. b) A system to control branch migration. The molecule shown contains four mobile nucleotide pairs (joined by open circles, rather than filled circles, like the other base pairs), and its horizontal arms are linked to form a circle. In the top molecule they are in the vertical arms, indicated by the orientation of the ellipse at the branch point. In the bottom molecule, they have relocated to the horizontal arms by the positive supercoiling action of ethidium; the ellipse has rotated. Primary cleavage sites for endonuclease VII are indicated by the large and small arrowheads. Note that they move relative to the sequence, but not the branch point. Labeled restriction sites for analysis are indicated, as is numbering within the fragments.

can be controlled by putting torque on one pair of opposite arms:[65] If the horizontal arms are negatively supercoiled, then the vertical arms will be extruded; if the horizontal arms are positively supercoiled, then the extrusion will be reversed.

Figure 21b illustrates a system where this feature has been used.[66] The horizontal arms are connected by a circle, in order to apply directional torque. The central four nucleotide pairs are symmetric, but that is the extent of the possible excursion of the branch point. Torque is applied by adding ethidium to the system. When this occurs, the four nucleotide pairs in the vertical arms relocate to the horizontal arms. This relocation is demonstrated by cleavage with endonuclease-VII, which cleaves at the branch point, and then by restriction with *Sca*I and *Eco*RV to analyze the cleaved fragment.[66] This system works, but it is cumbersome.

A more promising system entails utilizing the B→Z transition to produce a rotation between two objects. We have connected two DAO molecules with an appropriate length of DNA that can undergo the B→Z transition (proto-Z-DNA; Figure 22). When the molecule is under conditions

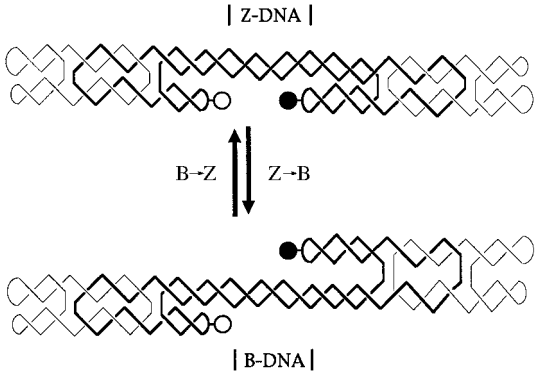

Figure 22. A nanomechanical device predicated on the B→Z transition. The molecule consists of two DAO molecules, connected by a segment containing proto-Z-DNA. The molecule consists of three cyclic strands, two on the ends drawn with a thin line, and one in the middle, drawn with a thick line. The molecule contains a pair of fluorescent dyes to confirm their separation by FRET. One is drawn as a filled circle, and the other as an empty circle. In the lower molecule, the proto-Z segment is in the B conformation, and the dyes are on opposite sides of the central double helix. In the top molecule, the proto-Z segment is in the Z conformation, and the dyes are on the same side of the central double helix. The length of the proto-Z-DNA and its conformation are indicated at top and bottom by the two vertical lines flanking the conformation descriptor.

that favor the B conformation, the unconnected domains of the DAO molecules are on opposite sides of the connecting domain. When the molecule is under conditions that favor the Z conformation, the unconnected domains are on the same side of the connecting domain. The transition is monitored by fluorescence resonance energy transfer (FRET) between two dye molecules. The system can be cycled between the two conditions, changing the structure of the molecules.[75] This is a relatively simple concept, but it has taken seven years to bring it to fruition, because the device requires rigid components to flank the proto-Z region. Only when the rigidity of DX molecules was established[42] was it possible to construct this device and demonstrate that it works.

6. Summary and Outlook

The aim of this review has been to demonstrate that the role of nucleic acids need not be limited to biological systems. The very properties that have allowed the process of natural selection to make such good use of the molecule as genetic material are the same ones that lead to a potentially valuable role in nanotechnology. The complementary relationship between the two opposite strands of DNA allows us to program its interactions to combine molecules in a desired fashion. This capability permits us to produce molecules with predictable topological properties. Thus, it has proved possible to build structures whose graphs have the connectivities of a cube or a truncated octahedron. Likewise, the linking topology of DNA is one of its most reliable properties; these geometrical constructions are precise catenanes of cyclic single strands of DNA. In the same manner, this topological control has enabled the construction of trefoil and figure-eight knots, along with the assembly of Borromean rings.

What is the future of this endeavor? There are several key issues that remain to be solved. The first of these is the construction of periodic matter. The recent assembly of two-dimensional arrays is an extremely encouraging development in this direction. Nevertheless, it remains to be shown that this success can be extended to three-dimensional systems. Furthermore, the currently successful assemblies are limited to simple DX molecules. Although we have successfully incorporated DX molecules into the edges of triangles, the triangles have not been assembled into periodic arrays yet. The greatest utility of this system is likely to be derived from arrays based on triangles or deltahedra; those arrays are most likely to accommodate large guest molecules within them, leading to the control of guest orientation within the host architecture.

The two-dimensional arrays that have been built do not have regular edges. Applications involving the use of such components to organize information will require the ability to address particular loci within an array. Well-defined edges are likely to be helpful to do this. Reif has suggested frames consisting of unique molecules in order to achieve this result.[67] Such assemblies are likely to be expensive, because each component of the frame must be unique.

Several of the goals outlined for DNA nanotechnology involve directing the spatial organization of other molecules by exploiting the architectural properties of DNA. It is easy to imagine chemical scenarios leading to this result, but little concrete progress has been made in this effort. Perhaps the most notable programmed attachment of a protein to DNA has been achieved by Smith et al., who placed specific cytosine methylases around a DNA molecule.[68] Further work must be done on the covalent attachment of organic molecules or polymers to DNA backbones.

Many research groups analyze the properties of DNA, and develop systems involving its hybridization and concatenation for bioanalytical purposes, but there has been little activity with structural objectives. Fortunately, this situation is changing. Recently, Mirkin et al. have attached DNA molecules to colloidal gold, with the goal of assembling nanoparticles into macroscopic materials[69] and with an eye toward diagnos-

tics.[70] Likewise, Alivisatos et al.[71] have used DNA to organize gold nanocrystals. Niemeyer et al.[72] had earlier used DNA specificity to generate protein arrays. In addition, Shi and Bergstrom[73] have attached DNA single strands to organic linkers. Nilsen, et al.[74] are working on another variation, DNA dendrimers.

DNA nanotechnology is a field poised for exploitation. The solid-support methodology appears to be a powerful means to assemble polyhedral objects from DNA. Likewise, DNA may be the most convenient system for the construction of topological targets. Although individual branched junctions are floppy, the DX molecule appears to be a DNA motif that has sufficient structural integrity that it can serve as the basis of periodic matter. Recent successes in the construction of two-dimensional periodic matter attest to this property. In addition, the use of DX molecules in a simple nanomechanical device supports the notion that this is the long-sought rigid component needed to exploit the properties of DNA as fully as they deserve. It is to be hoped that the advent of a rigid motif, linked with the useful properties exploited earlier will promote rapid progress in this field. Combined with the increase in activity from other laboratories, these developments suggest that DNA nanotechnology is likely to make major contributions in the twenty-first century.

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